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*J Immunol* 1998; 160:3163-3169;
http://www.jimmunol.org/content/160/7/3163

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Superdominance Among Immunodominant H-2K\textsuperscript{b}-Restricted Epitopes and Reversal by Dendritic Cell-Mediated Antigen Delivery\textsuperscript{1}

Johan K. Sandberg,\textsuperscript{2} Per Grufman, Elisabeth Z. Wolpert, Lars Franksson, Benedict J. Chambers, and Klas Kärrre

To examine possible interference patterns between immunodominant CTL Ags, we analyzed the response to mixtures of five well-characterized H-2K\textsuperscript{b}-restricted epitopes, each of which had earlier been described as immunodominant within its antigenic system. Clear patterns of dominance were observed between peptides in the mixture, with the CTL response focusing on the Sendai virus nucleoprotein 324–332 and vesicular stomatitis virus nucleoprotein 52–59 epitopes. The dominance of these epitopes correlated with high CTL availability. Subdominance of the OVA\textsubscript{257–264} and the MCF1233 murine leukemia virus envelope 574–581 peptides could not be explained by inferior ability to bind and stabilize MHC class I molecules. Interestingly, immunodominance was broken if the peptide mixture was pulsed on bone marrow-derived dendritic cells, a mode of immunization allowing efficient recognition of a broader set of specificities. Our results show that immunodominance is neither an absolute feature of a given epitope nor does it apply only in relation to other epitopes within the same protein, micro-organism, or cell. Novel “superdominant” hierarchies emerge in the response against multiple “dominant” epitopes. A T cell competition model to explain the data in terms of a balance influenced by CTL frequencies and available APC capacity is discussed. The Journal of Immunology, 1998, 160: 3163–3169.

C\textsuperscript{D8}\textsuperscript{+} T cells recognize 8- to 10-amino acid-long peptide fragments presented on the cell surface in complex with MHC class I molecules (1–5). The specific peptide ligands displayed are derived predominantly from intracellular proteins, enabling the CD8\textsuperscript{+} T cells to detect intracellular infection or malignant transformation (6). Specific MHC binding motifs (consensus motifs) that are associated with, but neither absolutely necessary nor sufficient for high affinity binding and CTL recognition, have been defined for class I-presented peptides, making prediction of CTL epitopes possible (7). The understanding of the central role of antigenic peptide fragments in the cell-mediated immune response has opened the possibility of using the corresponding synthetic peptides as vaccines (8), and there are numerous reports on induction of protective immunity against viruses (9–11) and tumors (12–16) using this approach.

Although many Ags carry several potential CTL epitopes, only one or a few are selected by the immune response in vivo, a phenomenon known as immunodominance (17). The major epitope(s) chosen by T cells in complex Ags are called immunodominant. Apart from the dominant epitope(s), complex Ags such as proteins, cells, or micro-organisms harbor a hierarchical array of subdominant and/or cryptic epitopes (18, 19). Both of these terms have been used operationally to define intrinsically immunogenic epitopes that fail to induce a response when introduced within a more complex antigenic challenge (17, 19, 21). According to a recent mechanistic classification proposed for class I-presented peptides, the term subdominant refer to epitopes that are processed and presented; they can elicit a response, but fail to do so in the presence of other “dominant” epitopes (22, 23). The term cryptic is then reserved for epitopes that are not presented. This may be accounted for by any of a number of mechanisms operating at the level of intracellular Ag processing. Peptide selection for cell surface expression can thus be influenced by proteasomal cleavage specificity (24–26) and protein sequences flanking the potential epitopes (27), as well as TAP-peptide transport preferences (28–31) and peptide competition for binding to the MHC class I molecule (21). The life span of MHC-peptide complexes, after they have arrived at the cell surface, influences the immunogenicity of potential epitopes (32). However, immunodominance can also be influenced by the available T cell repertoire (33–35) and the cytokine milieu (36).

A major limitation in the development of peptide vaccines is the extensive polymorphism in the human MHC loci. Additionally, immunity to antigenic variants and strains of pathogens may not be achieved using a single peptide. Thus, effective coverage of the human population will most probably need the inclusion of several epitopes. After good candidate peptides have been identified, the vaccine can be delivered loaded on APC, mixed with adjuvant, or as lipopeptides. Recently, peptides loaded on dendritic cells (DC)\textsuperscript{3} has proved particularly efficient in the induction of protective CTL immunity (12, 13).

To address the mechanisms of immunodominance in MHC class I-restricted T cell responses, as well as the functionality of multipetide peptide vaccines, we analyzed the CTL response to mixtures of five well-characterized H-2K\textsuperscript{b}-restricted epitopes, each of

\textsuperscript{1} This work was supported by grants from Arbetsmarknadens Försäkringsaktiebolag, the Swedish Cancer Society, and the Karolinska Institute.

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\textsuperscript{3} Abbreviations used in this paper: DC, dendritic cell; MCF, mink cell focus-inducing murine leukemia virus; mRas, mutant ras protein; SV, Sendai virus; VSV, vesicular stomatitis virus.
which has been described as immunodominant within its antigenic system (11, 37–41). Clear patterns of dominance among the five epitopes were observed, and the dominance was abrogated when DC were employed for Ag delivery. Our results have important implications for the understanding of immunodominance in T cell responses and for future vaccine development.

Materials and Methods

Mice and cell lines

C57BL/6 (B6) mice were bred and maintained at the Microbiology and Tumor Biology Center, Karolinska Institute, Stockholm, Sweden. Animal care was in accordance with institutional guidelines. Mice were used at 6 to 10 wk of age. The RMA-S cell line, a TAP2-deficient derivative of the Rauscher murine leukemia virus (MCF) 1233 env 574–581 KSPWFTTL (39); mutated Ras supematant. An 8-h51 Cr release assay was performed after 6 days, at peptide. Medium was supplemented with 20 IU/ml IL-2 and 5% MLC radiated with 2000 rad, and inoculated i.v. (46). 3) 13

Cells were then stained with FITC-conjugated anti-H-2Kb Ab AF6-88.5 dilution, together with 105 2000 rad-irradiated B6 splenocytes and 0.1 splenocytes were titrated in U-bottom 96-well plates using 60 wells at each

At 9 to 12 days after immunization with peptide in IFA, immune B6 splenocytes were titrated in U-bottom 96-well plates using 60 wells at each dilution, together with 103 2000 rad-irradiated B6 splenocytes and 0.1 μM peptide. Medium was supplemented with 20 IU/ml IL-2 and 5% MLC supernatant. An 8-h 51Cr release assay was performed after 6 days, at which time RMA-S target cells coated with peptide were added to 40 wells which time RMA-S target cells coated with peptide were added to 40 wells and RMA-S without peptide were added to 20 wells to measure the non-specific release. Positive wells were defined as those wells for which 51 Cr for 1 ha t37°C. Coated cells were labeled with 100 μl 1 nCi/ml 51Cr for 1 h at 37°C. Titrated numbers of restimulated effector cells were incubated with 3 × 105 51Cr-labeled target cells for 4 h at 37°C in 5% CO2. After incubation, released radioactivity was measured, and specific lysis was calculated according to the formula: % specific release = (experime ntal release – spontaneous release)/(maximum release – spontaneous release) 100.

Table I. Immunogenicity of H-2Kb-binding synthetic peptides

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Position</th>
<th>Sequence</th>
<th>Responding Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>SV NP</td>
<td>324-332</td>
<td>FAPGNYPAL</td>
<td>10/10</td>
</tr>
<tr>
<td>OVA</td>
<td>257-264</td>
<td>SIINFEKL</td>
<td>9/10</td>
</tr>
<tr>
<td>VSV NP</td>
<td>52-59</td>
<td>RGYVQYGL</td>
<td>9/10</td>
</tr>
<tr>
<td>MCF env</td>
<td>574-581</td>
<td>KSPWFTTL</td>
<td>6/9</td>
</tr>
<tr>
<td>mRas</td>
<td>59–67</td>
<td>AGLEEYSAM</td>
<td>2/8</td>
</tr>
</tbody>
</table>

* Fraction of in vivo-immunized mice giving peptide-specific lysis >10% as measured in a standard 4-h 51Cr release cytotoxicity assay after 5 days of restimulation in vitro.

**Immunodominance modulated by dendritic cells**

CTL assay

CTL activity was measured in a standard 51Cr release assay. Briefly, peptide-coated target cells were prepared by incubating cells with 30 μM of peptide for 1 h at 37°C. Coated cells were labeled with 100 μl 1 nCi/ml 51Cr for 1 h at 37°C. Titrated numbers of restimulated effector cells were incubated with 3 × 105 51Cr-labeled target cells for 4 h at 37°C in 5% CO2. After incubation, released radioactivity was measured, and specific lysis was calculated according to the formula: % specific release = (experimental release – spontaneous release)/(maximum release – spontaneous release) 100.

Results

Differential immunogenicity of five H-2Kb-restricted synthetic peptide epitopes

To examine the CTL response to multiple MHC class I restricted epitopes, five well-characterized peptide epitopes were chosen, each known to be H-2Kb restricted and immunodominant in the CD8+ T cell responses to their complex Ags (Table I). The VSV NP52, SV NP324, and MCF env574 are dominant in the CTL-mediated clearance of the corresponding viral infections, while OVA257 and mRas59 are in the focus of the CTL response to OVA and mutant Ras protein, respectively. To compare the magnitude of the CTL response to each of these five epitopes as single peptides, groups of B6 mice were immunized with the peptides in IFA. Immunogenicity was assessed as number of responding mice (Table I) and mean level of CTL activity in responding mice (Fig. 1). Three of these five epitopes, SV NP, VSV NP52, and the OVA257, reproducibly elicited strong CTL responses. The MCF env574 epitope induced an intermediate response, while in our hands, the mRas59 was only weakly immunogenic. The overall pattern was similar when mice were immunized with peptide-loaded live DC or irradiated splenocytes, although the DC route generally generated weaker CTL activity.

From the experiments shown in Table I and Figure 1 with mice immunized with single epitopes dissolved in IFA, or loaded on irradiated splenocytes or live DC, a hierarchical pattern of CTL reactivity is evident, with SV NP324, VSV NP52, and OVA257 being the most immunogenic, followed by MCF env574 and mRas59 in descending order.

Superdominance in the complex CTL response to five “immunodominant” synthetic peptide epitopes

With knowledge of the strength of the five CTL epitopes at hand, we wanted to investigate the simultaneous response to mixtures of these epitopes. When all five peptides were coinjected dissolved in adjuvant, the CTL response focused on the SV NP324 and VSV NP52 determinants; both of these epitopes elicited vigorous CTL responses (Fig. 2). The immunodominance of these two epitopes in...
the complex CTL response against the set of dominant epitopes was termed “superdominance.” The mRas<sub>59</sub>-specific response was weak but in the same range as against the single mRas<sub>59</sub> epitope (Fig. 2 and Fig. 1, respectively). Surprisingly, the OVA<sub>257</sub> and MCF env<sub>574</sub> epitopes now generated only very weak CTL responses, and we therefore termed these epitopes subdominant in the CTL response to the complex mixture.

Ag presentation by peptide-pulsed irradiated splenocytes inoculated i.v. generated a similar hierarchy in the CTL response (Fig. 2), i.e., the VSV NP<sub>52</sub> and SV NP<sub>324</sub> epitopes were dominant and OVA<sub>257</sub> and MCF env<sub>574</sub> were subdominant, while the mRas<sub>59</sub> epitope was virtually nonimmunogenic.

**Peptide administration on DC reverts superdominance**

Since bone marrow-derived DC have proved powerful in the delivery of MHC class I-restricted peptides for induction of immunity against tumors, for example (12, 13), we tested this mode of Ag delivery in relation to the superdominance among multiple dominant epitopes. B6 mice injected s.c. with DC pulsed with the five peptides responded vigorously to the SV NP<sub>324</sub>, VSV NP<sub>52</sub>, OVA<sub>257</sub> and MCF env<sub>574</sub> epitopes...
OVA 257, and MCF env 574 epitopes, while mRas 59 elicited only a very weak CTL response (Fig. 2). Thus, the subdominance of OVA 257 and MCF env 574 observed when the peptide mixture was injected in IFA or loaded on irradiated splenocytes was broken when peptides were presented in vivo, loaded on live DC.

The presence of several epitopes potentiate the CTL response against each Ag when loaded on DC

As shown above, the CTL response against the complex mixture of peptides dissolved in IFA focused on two epitopes, SV NP 324 and VSV NP 52. In most experiments, however, these dominant epitopes also elicited lower CTL activity in the mixture than when they were injected as single epitopes (Fig. 3, upper panel). In contrast, CTL responses against all peptides were boosted when the mixture was injected loaded on DC compared with single peptides loaded on DC (Fig. 3, lower panel). Thus, the DC route of Ag challenge not only reverted the subdominance of the OVA 257 and MCF env 574, but also enhanced CTL responses against all components in the complex mixture.

Differences in peptide affinity to H-2Kb do not correlate with dominance patterns but may explain the low immunogenicity of mRas 59

Since MHC class I binding affinity has proved to strongly influence peptide immunogenicity and determinant selection (21, 50, 51), we investigated whether the patterns of immunodominance observed in the peptide mixture correlated with differences in peptide affinity for class I. The stability of complexes between H-2Kb and each of the five peptides was tested by stabilizing “empty” cell surface Kb molecules on the TAP2-deficient cell line, RMA-S. Peptides were added to cultures of RMA-S maintained at 26°C overnight, followed by a 45-min chase at 37°C. The SV NP 324, OVA 257, VSV NP 52, MCF env 574, and mRas 59. The CTL activity against mRas 59 in IFA by CTL elicited against the mixture may represent a cross-recognition by SV NP 324-specific CTL, which has been observed in some experiments.

Differences in peptide affinity to H-2Kb do not correlate with dominance patterns but may explain the low immunogenicity of mRas 59

The frequency of specific T cells differs between dominant and subdominant peptide epitopes

The results described above suggested that dominance in the CTL response against the peptide mixture was not determined at the level of processing or binding to the restriction element, but rather at the T cell level. To address this possibility, we performed limiting dilution analysis with splenocytes from mice primed in vivo with the single peptides (Fig. 5). On days 9 through 12 after priming, the in vivo frequency of specific CTL differed markedly between the epitopes. The dominant epitopes SV NP 324 and VSV NP 52 both elicited CTL at a frequency ~1/25,000 splenocytes, while the OVA 257 and MCF env 574 frequencies were ~3.5-fold lower. Thus, the superdominance of the SV NP 324 and VSV NP 52 epitopes in the peptide mixture correlated with higher numbers of CTL at days 9 through 12 in the single peptide-immunized mice.

Dominant and subdominant CTL populations display generally similar avidity for their epitopes

T cell clones of different specificities may display different avidities for their specific ligands as an imprint of the selection process, which is likely to affect the specificities selected as dominant during a peripheral immune response. CTL generated against the single peptides were therefore tested against target cells loaded with titrated amounts of the specific peptides. CTL specific for the SV

FIGURE 3. Comparison of CTL responses against single and mixed peptides. The presence of several epitopes enhances the CTL responses when peptides are administered loaded on DC (lower panel), while dominance occurs when the IFA route of immunization is used (upper panel). Open bars represent CTL responses against the single epitopes, while filled bars represent responses against the mixture of SV NP 324, OVA 257, VSV NP 52, MCF env 574, and mRas 59. The CTL activity against mRas 59 in IFA by CTL elicited against the mixture may represent a cross-recognition by SV NP 324-specific CTL, which has been observed in some experiments.

FIGURE 4. Relative peptide affinity determined by H-2Kb cell surface stabilization. Flow cytometric analysis of peptide-induced stabilization of cell-surface H-2Kb on RMA-S. Data are expressed as relative fluorescence. One representative of four independent experiments is shown.
NP\textsubscript{324}, VSV NP\textsubscript{52}, OVA\textsubscript{257}, and MCF env\textsubscript{574} required similar doses of peptide (\textasciitilde 0.05 nM) for half-maximal lysis (Fig. 6, upper panel). Furthermore, these four CTL responses required similar overall amounts of peptide (\textasciitilde 1 nM) during the 5-day in vitro restimulation (Fig. 6, lower panel). T cells specific for SV NP\textsubscript{324} were activated at a somewhat lower peptide concentration both at the target cell level and during in vitro culture, suggesting a slightly elevated T cell avidity. However, there was no clear-cut difference between the dominant VSV NP\textsubscript{52} epitope and the subdominant OVA\textsubscript{257} and MCF env\textsubscript{574} epitopes.

**Discussion**

Immunodominance in T cells responses has been observed in several antigenic systems (11, 17–20, 22, 23, 37, 38). In the present study, immunodominance was found to occur in the complex CTL response against five synthetic peptides, each previously described as dominant in its own antigenic system. When the peptide mixture was delivered in adjuvant or loaded on splenocytes, the CTL response focused mainly on two epitopes, SV NP\textsubscript{324} and VSV NP\textsubscript{52}, while the OVA\textsubscript{257} and MCF env\textsubscript{574} epitopes were only very weakly recognized. The SV NP\textsubscript{324} and VSV NP\textsubscript{52} dominated among dominant epitopes; we use the term superdominance to describe this phenomenon. Most interestingly, when the five peptides were delivered on live DC, no superdominance was observed, and all four of these epitopes elicited similar CTL responses. Furthermore, when the DC route of immunization was employed, the presence of several epitopes potentiated the CTL responses against each epitope. The ability of DC to support efficient recognition of a broader range of epitopes can be valuable in the design of epitope-based vaccines.

Since the peptide epitopes were injected or loaded on APC in a preprocessed form, epitope selection at the level of intracellular Ag processing can be excluded as the cause of the observed immunodominance. Competition for binding to the MHC is a major factor influencing epitope selection and dominance patterns in CTL responses against complex Ags (21, 50, 51), although it is clear that peptide immunohierarchy in T cell responses does not always correlate with binding affinity to the restriction element (52). In the present study, the subdominance of OVA\textsubscript{257} and MCF env\textsubscript{574} could not be attributed to lower affinity to the MHC, since they stabilized H-2K\textsuperscript{b} equally as well as the dominant SV NP\textsubscript{324} and VSV NP\textsubscript{52}. A previous study indicated that SV NP\textsubscript{324}, OVA\textsubscript{257}, and VSV NP\textsubscript{52} have similar affinities for K\textsuperscript{b}, which supports this conclusion (53). The dominance patterns can probably be explained, therefore, by mechanisms operating at the T cell level. Indeed, the subdominance of both MCF env\textsubscript{574} and OVA\textsubscript{257} correlated with lower CTL frequency on days 9 through 12 after immunization, and that of MCF env\textsubscript{574} also correlated with slightly lower intrinsic immunogenicity. Peptide dose titrations both at the target cell level and upon restimulation in vitro suggest generally similar dosage requirements in dominant and subdominant responses, although a slightly higher T cell avidity for Ag was seen in the dominant SV NP\textsubscript{324}-specific response.
These results, together with the observation that delivery of Ags loaded on cultured DC could break dominance, are consistent with a model for immunodominance based on T cell competition for APC capacity. In this model, APC capacity is limiting in a competition between T cell clones. As a result of this competition, epitopes that are recognized by less frequent T cells may not be detected by a sufficient number of cells to elicit a response, even if these epitopes bind MHC molecules and are presented equally as well as Ags recognized by more frequent T cells. Inoculation of Ag loaded on 1 × 10^5 live DC could render APC capacity nonlimiting, since at a given time point after immunization, such mice would be likely to have a larger population presenting Ag in secondary lymphoid organs, compared with the natural recruitment of APC from the skin at the site of IFA injection. The differences in CTL frequencies were observed on days 9 through 12 after immunization and may reflect the number of T cell precursors before immunization, but we cannot exclude the fact that other factors influencing clonal expansion postimmunization contribute to the observed differences in CTL frequency as well as to the immunodominance patterns.

Cellular competition among CD8^+ T cells has been discussed in relation to several steps of T cell differentiation (54–57). Features of Ag presentation that could sharpen the competition and thus restrict diversity of T cell responses include numbers of APC, total APC surface available, and availability of cytokines. One good candidate cytokine that could regulate competition is IL-12. In fact, IL-12, recently shown to modulate immunodominance among HIV-1 epitopes (36), is produced by DC upon Ag-specific interaction with T cells (58) through ligation of cell surface CD40 (59). The latter observation can also explain our result that the presence of several epitopes on inoculated DC tend to potentiate responses against each Ag. Another candidate is TGF-β, since it can have both T cell-stimulatory (60) and -inhibitory (61) effects depending on the state of cell activation (62). Experiments to investigate the importance of cytokines in immunodominance are currently underway in our laboratory. We have recently observed immunodominance in a system based on minor histocompatibility Ags, in which events occurring postdeterminant selection appear to play a major role (our manuscript in preparation).

Our results show that immunodominance is not an absolute feature. Novel hierarchies emerge in the response against multiple dominant epitopes that do not necessarily reflect determinant selection within the APC. CTL responses against complex Ags harboring multiple epitopes that are equally well presented by the same MHC class I molecule may be balanced by differences in T cell availability and limitations in APC capacity.

Acknowledgments

The authors thank Dr. H. G. Ljunggren, Dr. R. Glas, and R. Wallin for helpful discussions.

References


